

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said method comprising:

(a) contacting said nucleic acid sequence, in one amplification reaction or separate amplification reactions, with a first pair of PCR primers and a second pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, a first member of said first pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said first pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said first pair hybridizes, and a first member of said second pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said second pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said second pair hybridizes, said PCR primers being characterized as follows:

(i) one of said first pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at a single nucleotide that is disposed

within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said first pair of primers is capable of amplifying said first allele under appropriate conditions; and

(ii) one of said second pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said second pair of primers is capable of amplifying said first allele under appropriate conditions;

(b) carrying out said amplification reaction or reactions, wherein the amplification reaction involving said first pair of PCR primers and the amplification reaction involving said second pair of PCR primers have different ranges of specificity; and

(c) detecting any amplification product of step (b), wherein the presence of amplification product is indicative of the presence of said first allele in said nucleic acid sequence.

2. (Cancelled)

3. (Currently Amended) The method of claim 1 [[2]], wherein said ranges of specificity overlap.

4. (Original) The method of claim 3, wherein said amplification reaction involving said first pair of PCR primers and said amplification reaction involving said second pair of PCR primers together have a greater than 3000-fold range of specificity.

5. (Original) The method of claim 4, wherein said amplification reaction involving said first pair of PCR primers and said amplification reaction involving said second pair of PCR primers together have at least a 10,000-fold range of specificity.

6. (Previously Presented) The method of claim 1, wherein said one of said second pair of PCR primers in step (a)(ii) includes at least two non-complementary nucleotides that are disposed within the five nucleotides adjacent to the 3'-terminal nucleotide of each primer.

7. (Previously Presented) The method of claim 1, wherein said polymorphic sequence comprises a single nucleotide polymorphism.

8. (Previously Presented) The method of claim 1, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) also comprise a universal primer binding site.

9. (Original) The method of claim 8, wherein said detecting step comprises amplification of said product of step (b) using a detectably labelled PCR primer that hybridizes to said universal primer binding site.

10. (Previously Presented) The method of claim 1, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) also comprise a unique hybridization tag.

11. (Cancelled)

12. (Previously Presented) The method of claim 10, wherein said detection step is carried out on a solid support to which a binding partner for each hybridization tag is immobilized.

13. (Original) The method of claim 12, wherein said solid support is a chip.

14. (Previously Presented) The method of claim 1, further comprising:

(d) contacting said nucleic acid sequence, in one amplification reaction or separate amplification reactions, with a third pair of PCR primers and a fourth pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, a first member of said third pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said third pair of PCR primers

hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said third pair hybridizes, and a first member of said fourth pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said fourth pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said fourth pair hybridizes, said PCR primers being characterized as follows:

(i) one of said third pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said third pair of primers is capable of amplifying said second allele under appropriate conditions; and

(ii) one of said fourth pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said fourth pair of primers is capable of amplifying said second allele under appropriate conditions; and

(e) carrying out said amplification reaction or reactions; and

(f) detecting any amplification product of step (e), wherein the presence of amplification product is indicative of the presence of said second allele in said nucleic acid sequence.

15. (Currently Amended) A kit for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said kit comprising:

(a) a first pair of PCR primers and a second pair of PCR primers, a first member of said first pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said first pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said first pair hybridizes, and a first member of said second pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said second pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said second pair hybridizes, wherein said PCR primers are characterized as follows:

(i) one of said first pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said first pair of primers is capable of amplifying said first allele under appropriate conditions; and

(ii) one of said second pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said second pair of primers is capable of amplifying said first allele under appropriate conditions, wherein an amplification reaction involving said first pair of PCR primers and an amplification reaction involving said second pair of PCR primers have different ranges of specificity.

16. (Previously Presented) The kit of claim 15, further comprising:

(b) a third pair of PCR primers and a fourth pair of PCR primers, a first member of said third pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said third pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said third pair hybridizes, and a first member of said fourth pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said fourth pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said fourth pair hybridizes, wherein said PCR primers are characterized as follows:

(i) one of said third pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said third pair of primers is capable of amplifying said second allele under appropriate conditions; and

(ii) one of said fourth pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at one or more nucleotides that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said fourth pair of primers is capable of amplifying said second allele under appropriate conditions.

17. (Previously Presented) The kit of claim 15, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) also comprise a universal primer binding sequence.

18. (Previously Presented) The kit of claim 15, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) also comprise a unique hybridization tag.

19. (Original) The kit of claim 18, wherein said kit further includes a solid support to which is immobilized a binding partner for each hybridization tag.

20. (Original) The kit of claim 19, wherein said solid support is a chip.